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TAXONOMIC STUDY OF THE CLASS ULVOPHYCEAE (CHLOROPHYTA) FROM CERTAIN AREAS OF THE PUNJAB, PAKISTAN

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Abstract: Fifteen species of the grass-green algae belonging to the genera *Aphanochaete, Chaetophora, Coleochaete, Cylindrocapsa, Microspora and Stigeoclonium* were collected from various freshwater habitats in Gujranwala, Jhang, Kasur, Lahore, Sheikhupura and Sialkot Districts of the Punjab Province (Pakistan) during April 2004 and May 2005. They were taxonomically determined and found to belong to the orders Prasiolales, Microsporales, Chaetophorales and Coleochaeteales. They have been described for the first time from these areas.

Keywords: Algae, Chlorophyta, *Aphanochaete, Chaetophora, Coleochaete, Cylindrocapsa, Microspora, Stigeoclonium,* taxonomy

Introduction

Following the classical pioneering work conducted by Randhawa [1] on the taxonomy of grass-green algae from the area of the Punjab only limited studies have been carried out on this aspect [2-6]. The present research work on the algal flora of this area of the Punjab was done in view of the paucity of available literature on the subject. Starting from December 2003, a large collection of green algae was made from fresh-water habitats of various districts of the Punjab, certain areas of NWFP and Azad Kashmir for syatematic taxonomic studies [7-10]. The present paper is a continuation of that research program. Presently, taxonomy of 15 species of the class Ulvophyceae has been described.

Materials and Methods

The specimens of Ulvophyceae were collected by hand-picking from various freshwater habitats (slow running water, sides of stagnant ponds, paddy fields, streams) in Gujranwala, Jhang, Kasur, Lahore, Sheikhupura and Sialkot Districts of the Punjab Province (Pakistan) during April 2004 and May 2005. They were carefully washed, preserved in glass bottles containing 4% formalin, brought to the laboratory at Karachi and taxonomically investigated as described earlier [7]. The collected material was identified under light microscope (Olympus, Japan; objective 20 x, eye-piece 10 x) with the help of authentic literature [11-22]. Diagrams were made with the help of camera lucida and the voucher specimens were kept in the Phycology & Phycochemistry Lab., MAH Qadri Biological Research Centre, University of Karachi, where this research work was conducted.

Results and Discussion

Fifteen species of the grass-green algae belonging to the phylum Chlorophyta, Class Ulvophyceae, orders Prasiolales, Microsporales, Chaetophorales and Coleochaetales have been identified and arranged according to the recently proposed classification [23]. Their taxonomic enumerations are given below.

Order: **Prasiolales**

Thallus foliose; cells with stellate chloroplast [23]. Details are given in the family characters.

Family: **Cylindrocapsaceae**

Thallus filamentous, unbranched; oblong or cylindrical, uninucleated cells; surrounded by tough, pectose, often lamellated, mucilaginous sheath; in older portions or under certain conditions of growth cells often irregularly arranged in a pseudoparenchymatous manner due to the division of cells in more than one plane and formation of septa oblique or diagonal to the longitudinal axis; cell wall several layer thick, refractive, mostly made of cellulose; chloroplast massive, without any distinct patern or stellate, axial, each with a central pyrenoid; vegetative reproduction by fragmentation, asexual reproduction by bi- or quadriflagellate zoospores, sometimes replaced by aplanospores; small zoospores called androspores; sexual reproduction oogamous, filaments homothallic or heterothallic. The present collection included the following genus.

Cylindrocapsa Reinsch 1867

Unbranched filamentous thalli; oblong-ellipsoid, cylindrical or short, quadrate cells, arranged mostly uniseriately, but rarely biseriate, multiseriate or in irregular portions; vegetative reproduction by fragmentation, asexual reproduction by biflagellate zoospores, sexual reproduction oogamous. Only the following species was included in the present collection.

1. C. involuta Reinsch 1867, References: [11: 83, 12: 141].

Morphological characters: Thallus polymorphic, filaments well formed, uniseriate, cylindrical, unbranched (Fig.1), but portions appearing multicellular and parenchymatous (Fig. 2).

Cytological features: Cylindrical, constricted at cross walls, 8-9 µm broad and 5-9 µm long; cell-wall thick, hyaline; chloropasts massive, with a single or many pyrenoids.

Reproductive structures: Filaments dioecious; oogonia formed by enlargement of one or more cells (Fig. 3), oogonia globose or pyriform, 9-12 µm broad and 11-13 µm long, dark reddish brown in colour; antheridia one or more in a row, formed by the repeated division of cell contents in two, four eight or sixteen protoplasts (Fig. 4), antheridia 13-17 µm broad and 13-17 µm long.

Locality: Sheikhupura District: Faizpura Village (30-4-2004), Sialkot District: Darganwali Village (25-5-2004).

Geographical distribution: Previously reported from Europe and India [12].

Remarks: The observed specimens were collected from ponds as free floating, entangled masses and from roadside puddles during early summer.

***Order*: Microsporales**

Filamentous thallus; cell-walls composed of H-pieces [23]. Details are given in the family characters.

***Family*: Microsporaceae**

Unbranched, filamentous thalli, found mostly in freshwater; cells cylindrical or slightly barrel shaped; cell-wall made up of "H-shaped" pieces, each protoplast enclosed by overlapping halves of two successive H-shaped pieces; chloroplast varies with age and species, may be parietal plate, perforated or apparantly reticulate; pyrenoids absent; nucleus large, distinct and centrally placed. This family includes the following single genus which was collected.

Microspora Thuret 1850: 221

Filaments evidently sessile when young, but

mostly free floating when mature; cells cylindrical or slightly barrel shaped; cell-wall nearly homogenous in appearance in some species, but in most cases composed of H-pieces, sometimes heavily impregnated with wall material and appear striated; asexual reproduction by aplanospores, biflagellated or quadriflagellated zoospores; sexual reproduction not known. The following species of this genus were collected, which may be distinguished as follows:

1. Cells constricted at the cross wall..... 2
   Cells not constricted at the cross wall
   ..... *M. wittrockii (6)*
2. Cells always constricted at the cross wall
   ..... *M. tumidula (5)*
   Cells seldom constricted at the cross wall.3
3. Cells more than 15 µm broad
   ..... *M. loefgrenii (3)*
   Cells less than 15 µm broad 4
4. Cells up to 24 µm long .... *M. floccosa (2)*
   Cells less than 24 µm long
   ..... *M. tenerrima (4)*

2. M. floccosa (Vaucher 1803) Thuret 1850: 221, References: [11: 101, 12: 120, 13: 107, 20: 269].

Basionym: Prolifera floccosa Vaucher 1803.

Synonym: Lyngbya floccosa (Vaucher) Hassall 1845.

Morphological charaters: Filaments unbranched, light blue in colour, on fragmentation disassociate into H-pieces (Fig. 5).

Cytological features: Cells generally cylindrical, seldom constricted at the cross walls, 23-24 µm long and 13-15 µm broad; cell-wall in two sections, overlapping in mid regions as indicated by broken ends of filament where section of terminal cell protrudes.

Reproductive structures: Thick walled akinetes.

Locality: Lahore District: Ghulam Colony (16-7-2004).

Type Locality: Near Geneva, Switzerland.

Geographical distribution: Widely distributed in North America, Europe, Asia (including India), Africa, Australia and New Zealand [12].

Remarks: The collection was made from paddy fields in summer where it occurred in massive growth and free floating condition.

3. M. loefgrenii (Nordstedt 1882) Lagerheim 1887: 417, References: [12: 130, 13: 107, 21: 349].

Basionym: Conferva loefgrenii Nordstedt 1882.

Morphological characters: Unbranched filaments (Fig. 6).

Cytological features: Cells slightly swollen, short, cylindrical or rectangular, 15-17 µm broad and two times as long as broad; H-pieces clearly seen in the mid region; chloroplast net like, nearly covering the entire cell lumen.

Reproductive structures: Asexual reproduction by thick walled aplanospores.

Locality: Jhang District: Trimmu Head Works (22-1-2004).

Geographical distribution: Previously reported from Europe, Asia, Africa, North and South America.

Remarks: It was collected during spring, occurring in ponds mixed with other algae.

4. M. tenerrima (Kützing) Gay, Reference: [12: 136].

Morphological characters: Thallus unbranched

filaments, on fragmentation dissociate into H-pieces (Fig. 7).

Cytological features: Cells short, cylindrical, 13-15 μm long and 6-7 μm broad; chloroplast varies in appearance either a parietal, folded, discontinuous plate or a mesh work of strands; cell-walls in two sections overlapping in the mid region, as indicated by the broken ends of the filaments where the section of the terminal cell protrudes.

Reproductive structures: In the present collection reproductive structures were not observed.

Locality: Lahore Districts: Lakho Dahr (10-12-2004).

Geographical distribution: Cosmopolitan.

Remarks: The specimens were collected from a temporary pond near Lakho Dahr, Lahore during winter. They were found in low quantity because the temperature was not favourable for their growth.

5. M. tumidula Hazen 1902: 177, References: [12: 122, 13: 108, 14: 447, 19: 545].

Morphological characters: Unbranched filamentous form.

Cytological features: Cells nearly cylindrical, slightly constricted at cross walls, 7-8 μm broad and 6.0-11.5 μm long; cell-wall thin; chloroplast dense, perforated, covering large portion of the cell lumen.

Reproductive structures: Vegetative reproduction by akinetes, 8-11 μm thick, rounded or somewhat flattened, formed singly in each cell, released by a break up of filaments into H-pieces (Fig. 8); aplanospores produced by division of protoplast (Fig. 9).

Locality: Kasur District: 15 km away from Kasur (28-1-2004); Sialkot District: Darganwali Village (25-5-2004).

Geographical distribution: Recorded in brooks and stagnant waters of Europe, Asia, Africa, North & South America and India [12].

Remarks: It was collected from two different localities of the Punjab during spring and summer. It was found in roadside puddles and temporary ponds, mixed with *Uronema confervicola* Lagerheim.

6. M. wittrockii (Wille 1881) Lagerheim 1887, References: [12: 130, 21: 349].

Basionym: Coferva wittrockii Wille 1881.

Morphological characters: Unbranched filaments (Fig. 10).

Cytological features: Cells cylindrical, not constricted at cross walls, 22-24 μm broad and 33-35 μm long; chloroplast in the form of thin sheet with perforations.

Reproductive structures: Aplanospores and hypnospores.

Locality: Sheikhupura District: near Sattarwala Village (30-8-2004).

Geographical distribution: Asia, Africa, Europe, North and South America [24].

Remarks: The collection was made during late summer from bank of a canal. The climate of Sheikhupura District is of semi-arid type. The soil at the place of collection was made up of silt, clay and large proportion of sand with pH 8 *i.e.* slightly alkaline.

***Order:* Chaetophorales**

Simple or branched, heterotrichous filaments; cells with plasmodesmata [23]. Details are given in the family characters.

Family: **Chaetophraceae**

Branched filaments with basal-distal differentiation; branches terminate in sharp point or in setae of several cells in length; found in both fresh and marine waters; thalli enclosed in either firm and tough or in soft and amorphous mucilage; two general plane of growth, some have prostrate development which may or may not give rise to erect branches, others develop into erect thalli with little or no prostrate portion, but may have downwardly directed, rhizoidal branches; reproduction by zoospores and sexuality by isogametes. The following two genera of this family were collected, which may be distinguished as follows:

1. Filaments poorly branched, enclosed in a thin mucilage ..... *Stigeoclonium*
   Filaments much branched, enclosed in firm, copious mucilage ..... *Chaetophora*

Chaetophora F. Schrank 1783: 125

Thallus consisting; of highly branched filaments, arising from a prostrate, palmelloid mass of cells; enclosed by mucilage of such a firm consistency as to give the thallus a definite shape as globose, hemispherical or arbuscular; branches tapering to either a blunt point or a long, multicellular, hyaline hair; chloroplast a parietal band, in the upper cells completely covering the lateral walls, with one or more pyrenoids; zoospores and isogametes formed in the outer cells of the branches. Present collection included the following species.

7. C. attenuata *Hazen* 1902: 213, References: [13: 118, 22: 104].

Morphological characters: Thallus richly branched; branches radiating out from a common center; embedded in compact mucilage to form smaller and larger globules (Fig. 11).

Cytological features: Terminal cells of branches prolonged into long colourless hairs; cells 5-6 µm broad and 18-19 µm long.

Reproductive structures: Reproductive structures were not observed.

Locality: Lahore District: Lakho Dahr (3-10-2004).

Geographical distribution: Previously reported from U.S.A., Michigan.

Remarks: The collection work was done during autumn in the freshwater pond. The specimens were collected in vegetative stage, being epiphytic on sub-merged grasses and water plants.

Stigeoclonium *Kützing* 1843

Thalli heterotrichous, prostrate portion more developed than the erect portion; coated with soft, inconspicuous mucilage; erect portion branched, branches mostly opposite or alternate according to species, ending in sharp points or taper to setae; cells cylindric or tumid; chloroplast parietal plate with a pyrenoid; some species have downwardly growing branches with irregularly thickened walls; asexual reproduction by bi- and quadriflagellated zoospores. The following four species of this genus were collected, which may be distinguished as follows:

1. Branching alternate 2
   Branching opposite 3
2. Filaments short, tufted ..... *S. nanum (10)*
   Filaments elongated ..... *S. elongatum (8)*
3. Cells more than 19 µm long *S. tenue (11)*
   Cells less than 19 µm long *S. lubricum (9)*

8. S. elongatum (Hassall 1843) Kützing 1849, References: [13: 115, 15: 104].

Basionym: Draparnaldia elongata Hassall 1843: 428.

Morphological characters: Thalli alternately branched with elongated filaments, 5.5-7.6 µm broad; ultimate branches gradually tapered (Fig.12).

Cytological features: Vegetative cells 5.5-7.6 µm broad; terminal cells modified into setae.

Reproductive structures: Reproductive structures were not observed.

Locality: Lahore District: fountain of Minar-e-Pakistan (10-4-2004), Handoo Village (5-5-2004), Mahmood Booti (2-7-2004); Sialkot District: near Ravi Marala Link Sambraal Road (6-4-2004).

Geographical distribution: Previouly reported from Asia, America, Europe and New Zealand.

Remarks: It was collected during early, mid and late summer from three different places of the Punjab. It was found growing massively in stagnant water pools, paddy fields, tube well houses, roadside puddles and fountain of historical places.

9. S. lubricum (Dillwyn 1809) Kützing 1843: 1898, References: [6: 108, 13: 115, 15: 118].

Basionym: Coferva lubrica Dillwyn 1809: 57.

Morphological characters: Thallus with prostrate and erect portions (Fig. 13).

Cytological features: Cell-wall thick; terminal cells modified into setae, vegetative cells 10-13 µm broad and 15-19 µm long.

Reproductive structures: Reproductive organs were not observed.

Locality: Gujranwala District: Nandipur (19-2-2004), Singh Village (11-12-2004); Lahore District: fountain of Badshahi Mosque (3-4-2004), Handoo Village (4-3-2004), Mahmood Booti (2-7-2004); Kasur District: Galwedah (11-3-2004); Jhang District: Rabwah: (15-8-2004).

Geographical distribution: Previously known from U.S.A. and other countries.

Remarks: It was collected from different areas of the Punjab during spring, summer, autumn and winter seasons. It occurred in a variety of habitats such as discharge box of tube well, fountains, paddy fields, canal ponds, roadside puddles and stagnant water ponds, being epiphytic on submerged grasses.

10. S. nanum (Dillwyn 1809) Kützing 1849: 354, References: [13: 116, 15: 59].

Basionym: Conferva nanum Dillwyn 1809.

Morphological characters: Thallus of short tufted filaments; branches arising alternately and tapering to a blunt point (Fig. 14).

Cytological features: Cells of branches scarcely smaller than those of main axis, 9-11 µm broad, those of branches 4-9 µm broad; chloroplast parietal, reticulate; hair forming cells colourless; posterior portion extensively pseudoparenchymatous or filamentous; cells sub-globose, giving rise to vertical branches.

Reproductive structures: Reproductive structures were not observed.

Locality: Gujranwala District: Dera Ahmad Shah Wirk (11-12-2004), Nandipur (19-2-2004).

Geographical distribution: Previously reported from U.S.A. and other countries of the world.

Remarks: It was collected during winter and spring. It was found in ponds on submerged plants (epiphytic along with *Aphanochaete* spp.) and in canalside ponds.

11. S. tenue (C. A. Agardh 1824) Kützing 1843: 253, References: [13: 117, 15: 90, 16: 247, 17: 55, 18: 95, 19: 548, 22: 105].

Basionym: Draparnaldia tenuis C. A. Agardh 1824: 34.

Synonym: Myxonema tenue (C. A. Agardh) Rabenhorst.

Morphological characters: Filaments with opposite branching; ultimate ends tapering (Fig 15).

Cytological features: Lower cells cylindrical, 9.0-12.5 µm broad and 23.0-43.5 µm long.

Reproductive structures: Reproductive structures were not observed.

Locality: Lahore: fountain of Minar-e-Pakistan (6-4-2004); Sheikhupura District: between Narang Mundi and Mureedke (20-9-2003); Sialkot District: near Bajra Village (25-5-2004).

Geographical distribution: Previously reported from India and U. S. A. [22].

Remarks: The specimens were collected during the month of summer and autumn. It occurred in fountains, paddy fields and permanent ponds, mixed with *Spirogyra* spp.

Order: **Coleochaetales**

Branched filaments or discoid thalli; sheathed setae present; oogamous sexual reproduction; motile cells have a covering of scales [23]. Details are given in the family characters.

Family: **Aphanochaetaceae**

Unicellular or pseudo-filamentous, prostrate or creeping thalli; chloroplast a parietal plate; cells with various types of bristles or hairs; hairs arise either from the wall or from the protoplast and protrude through a pore in the wall, some hairs have a sheated base; generally oogamous, with certain cells forming oogonia or antheridia. The present collection included only the following genus:

Aphanochaete A. Braun 1851: *andot.*

Epiphytic on various aquatic plants; thalli prostrate or with slightly erect open branches; each cell has a parietal chloroplast with pyrenoids; some cells bear fragile hairs, which are easily broken off; reproduction by qudriflagellated zoospores; sexual reproduction isogamous, gametes qudriflagellate. The following two species of this genus were collected, which may be distinguished as follows:

1. Cells spherical ..... *A. repens (13)*
   Cells rounded or slightly rectangular
   ..... *A. polychaete (12)*

12. *A. polychaete* (Hansgirg) F. E. Fritsch 1902: 411

Basionym: Herposteiron polychaete Hansgirg.

Morphological characters: Thallus sparsely branched, creeping or filamentous (Fig. 16).

Cytological features: Cells rounded or slightly rectangular, 13-14 µm broad and 14-16 µm long; septa arising from the dorsal wall of each cell.

Reproductive structures: Reproductive structures were not observed.

Locality: Gujranwala District: Nandipur (19- 2-2004).

Geographical distribution: Worldwide.

Remarks: It was collected during spring and was found in a canalside pond, epiphytic on submerged plants.

12. A. repens A. Braun 1850: 196, References: [4: 351, 14: 447, 18: 97].

Synonyms: Aphanochaete confervicola (Nägeli *ex* Kützing) Rabenhorst; *Herposteiron repens* (A. Braun) Wittrock; *Gonatoblaste rostrata* Huber.

Morphological characters: Filaments creeping; setae borne singly on mature cells, up to 100 µm long (Fig. 17).

Cytological features: Cells almost spherical, 5-10 µm broad and 8-16 µm long, constricted; each with parietal plate like chloroplast.

Reproductive structures: Reproductive organs were not observed.

Locality: Gujranwala District: Nandipur (12-1-2004).

Geographical distribution: Previously reported from Europe and U.S. A.

Remarks: Collections have been made during spring. It was found in a stagnant water pond, mixed with other free floating filamentous algae like *Cladophora* spp.

Family: **Coleochaetaceae**

The thallus either cushion-shaped or disc-like; some cells produce setae with a sheath at the base; thalli mostly epiphytic; reproduction by zoospores, sexual reproduction oogamous.

Coleochaete *de* Brébisson 1844: 29

Thallus much branched and orbuscular, closely appeared to substratum; sometimes discoid due to marginal cell division in a radial and tangential direction; each cell contains a parietal, luminate chloroplast with pyrenoids; some cells bear setae, each with a basal sheath or collar; sheath and hairs delicate and readlilly break off near the base; asexual reproduction by biflagellated zoospores, produced singly by cells, aplanospores also produced; sexual reproduction oogamous; female reproductive cell has a trichogyne; antheridia small cells, each producing a biflagellated spermatozoid. The following two species of this genus were collected, which may be distinguished as follows:

1. Cells oblong to pyriform
   ..... *C. pulvinata (14)*
   Cells irregularly polygonal
   ..... *C. scutata (15)*

13. C. pulvinata A. Braun *in* Kützing 1849: 425, Reference: [13: 129].

Morphological characters: Thallus hetero-trichous, forming a cushion of irregularly branched filaments, radiating from a common center (Fig.18).

Cytological features: Cells oblong to pyriform, somewhat inflated anteriorly, 10-12 µm long and almost as broad as long.

Reproductive structures: In the present specimens reproductive structures were not seen.

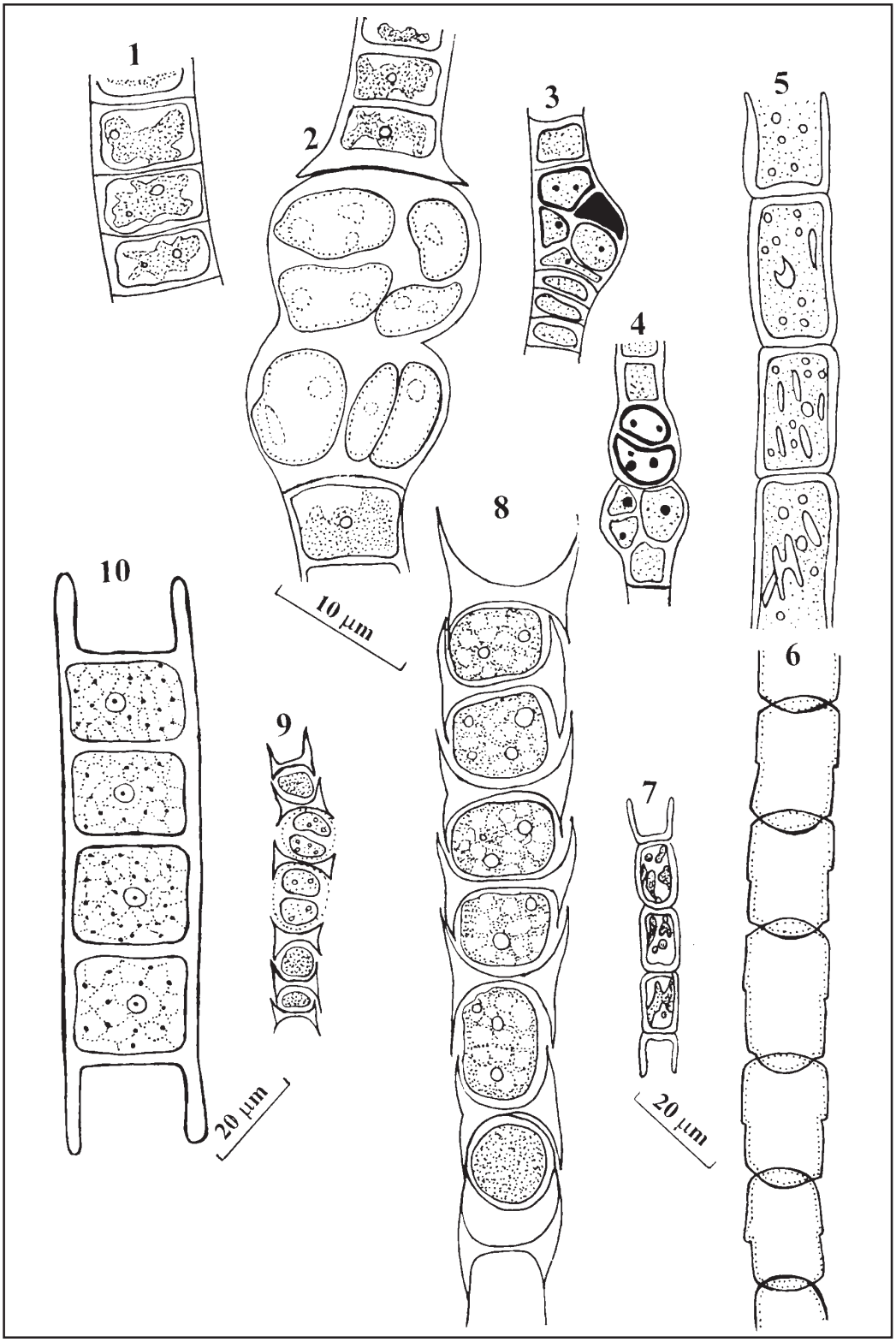
Locality: Lahore District: Mahmood Booti (24-5-2005).

Geographical distribution: Worldwide.

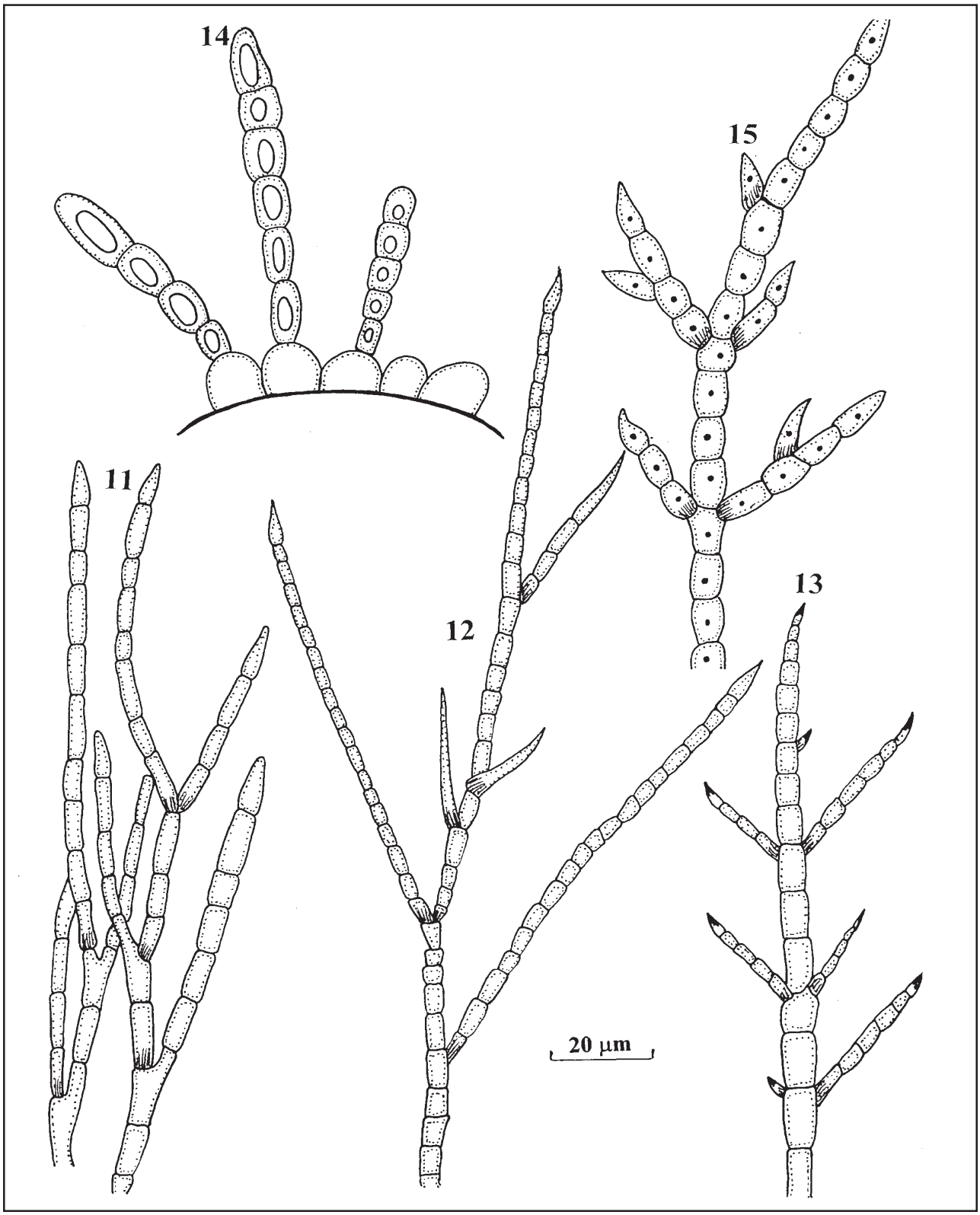
Remarks: The observed specimens have been collected during summer. They were epiphytic on the culm of *Paspalum distichum* in high quantity in paddy fields.

14. C. scutata de Brébisson 1844: 29, References: [6: 109, 11: 69, 13: 130, 16: 250, 19: 551].

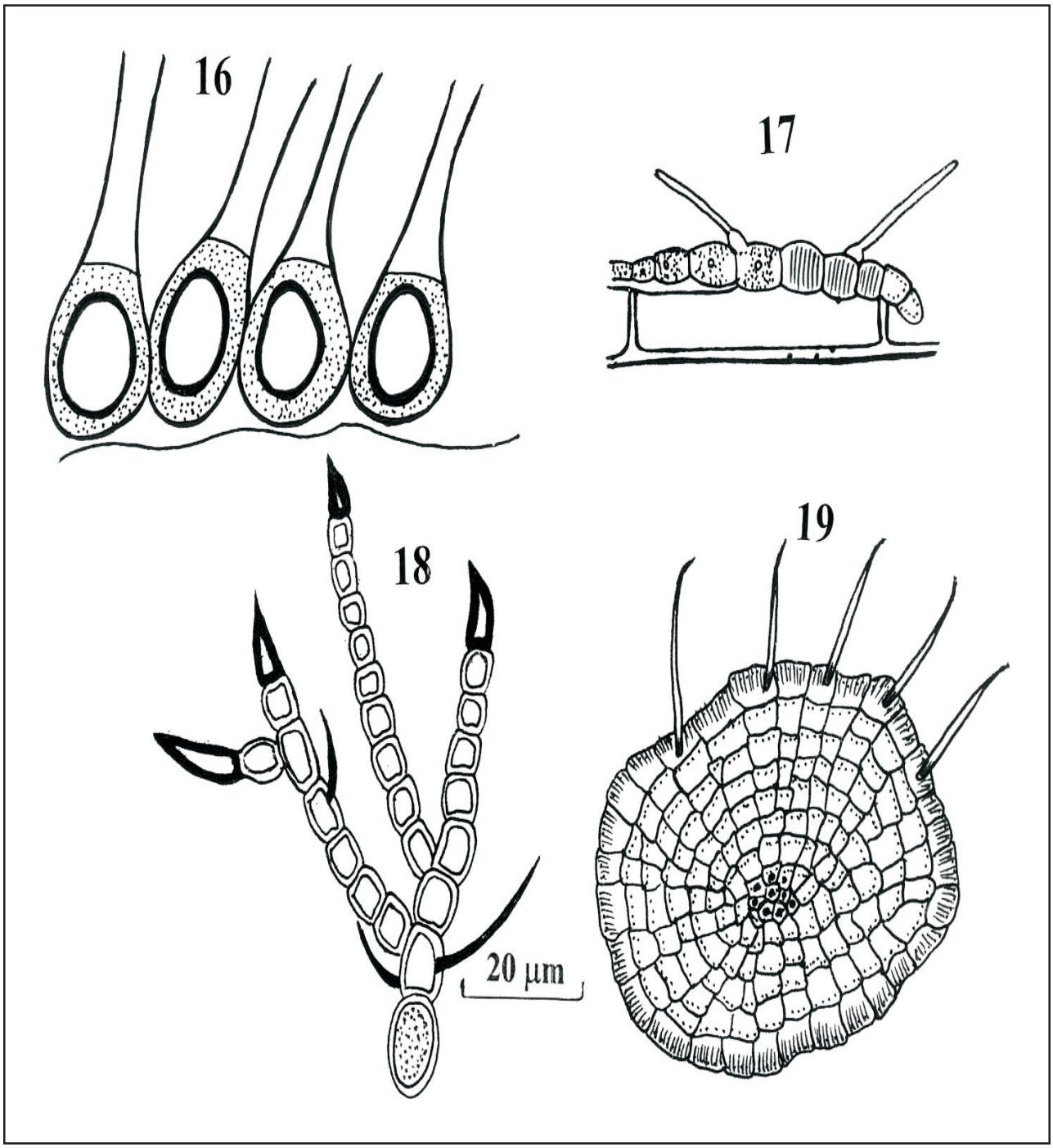
Morphological characters: Thallus flat, 54-59 µm in diameter, formed from lateral fusion of radiating, branching filaments, irregular in outline (Fig. 19).



Figs. 1-10. Species of *Cylindrocapsa* and *Microspora*: 1. *Cylindrocapsa involuta,* uniseriate filament, 2. *C. involuta,* becoming parenchymatous, 3. *C. involuta,* oogonium formation, 4. *C. involuta,* antheridium formation, 5. *Microspora floccosa,* 6. *M. loefgrenii*, 7. *M. tenerrima,* 8. *M. tumidula*, akinete formation, 9. *M. tumidula*, aplanospores formation, 10. *M. wittrockii*.



Figs. 11-15. Species of *Chaetophora* and *Stigeoclonium*: 11. *Chaetophora attenuata*, 12. *Stigeoclonium elongatum*, 13. *S. lubricum*, 14. *S. nanum*, 15. *S. tenue*.



Figs. 16-19. Species of *Aphanochaete* and *Coleochaete*: 16. *Aphanochaete polychaete*, 17. *A. repens*, 18. *Coleochaete pulvinata*, 19. *C. scutata*.

Cytological features: Cells irregularly polygonal, some with erect setae; vegetative cells 3-4 μm in diameter.

Reproductive structures: In the present collection reproductive structure were not observed.

Locality: Jhang Districts: Rabwah (21-3-2004).

Geogrophical distribution: Previously reported from USA: Michigan and Wisconsin.

Remarks: The collections were made along the bank of river Chenab near Rabwah during early summer. It was found in massive quantity and in free floating state.

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